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ORIGINAL RESEARCH PAPER

Modification of alternan by dextranase

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Abstract Alternan is a unique glucan with a backbone structure of alternating α-(1 → 6) and α-(1 → 3) linkages. Previously, we isolated strains of *Penicillium* sp. that modify native, high molecular weight alternan in a novel bioconversion process to a lower molecular weight form with solution viscosity properties similar to those of commercial gum arabic. The mechanism of this modification was unknown. Here, we report that these *Penicillium* sp. strains secrete dextranase during germination on alternan. Furthermore, alternan is modified in vitro by commercial dextranases, and dextranase-modified alternan appears to be identical to bioconversion-modified alternan. This is surprising, since alternan has long been considered to be resistant to dextranase. Results suggest that native alternan may have localized regions of consecutive α-(1 → 6) linkages that serve as substrates for dextranase. Dextranase treatment of native alternan, particularly with GRAS enzymes, may have practical advantages for the production of modified alternan as a gum arabic substitute.

Keywords Alternan · Dextranase · Gum arabic · Modified alternan · Viscosity

Introduction

Alternan is a unique glucan with a backbone structure of alternating α-(1 → 6) and α-(1 → 3) linkages (Misaki et al. 1980; Cote and Robyt 1982). Alternan exhibits properties of high solubility and relatively low viscosity as well as resistance to most known microbial and mammalian enzymes (Cote 1992, 2002). Native alternan has an apparent weight-average molecular weight of 10^6–10^7 (Cote 1992; Leathers et al. 2002a). Previously, we isolated novel strains of *Penicillium* sp. that modify alternan to lower apparent molecular weight forms with rheological properties that more closely resemble those of gum arabic (Leathers et al. 2002a, b, 2003, 2006). Gum arabic is used in the preparation of confectionaries, beverages, encapsulated flavors, and pharmaceuticals (Whistler 1993; Williams and Phillips 2000). The price, quality, and availability of gum arabic vary considerably, and it would be desirable to have a domestic substitute of consistent quality.

U.S. Department of Agriculture—Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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The modification of alternan by *Penicillium* sp. strains is a novel bioconversion process. Spores from these strains are germinated in medium containing alternan as a sole carbon source. The fungi exhibit only limited growth on alternan and do not consume the substrate, resulting in a near-quantitative bioconversion of native to modified alternan. Prior to the current study, the mechanism of this bioconversion process was unknown. *Penicillium* sp. culture supernatants, cell suspensions and cell extracts were devoid of measurable alternanase enzyme activity. Although *Penicillium* is well known as a source of dextranase, alternan has long been considered to be resistant to endodextranase activity (Cote 1992).

Materials and methods

Polysaccharides and enzymes

Native alternan was produced as previously described from *Leuconostoc mesenteroides* strain NRRL B-21297 (Leathers et al. 1995, 1997a, b). Bioconversion-modified alternan was produced using novel strains of *Penicillium* sp. as previously described (Leathers et al. 2002a, b). Specifically, a basal liquid medium ("WW") of Koenig and Day (1989) containing 1% (w/v) native alternan was inoculated to 10^5 spores/ml and incubated at 28°C and 200 rpm for 7 days. Commercial dextranases were from *Penicillium* sp. (Sigma) or from *Chaetomium erraticum* (Bio-Cat Inc., Troy, VA).

Analytical methods

The A_{225} of appropriate dilutions of alternan solutions was monitored as a measure of alternan modification (Leathers et al. 2002a). Polysaccharide molecular weight distributions were analyzed by size exclusion chromatography using a Shodex SB-806 M HQ column (Showa Denko, Tokyo, Japan) eluted with 0.05 M sodium nitrate at room temperature at 0.5 ml/min. Separations were monitored using a Shodex OR-1 optical rotation detector (Showa Denko K.K.). Dextranase activity was measured using a reducing sugar assay with 1 U representing the release of 1 μmol isomaltose (measured as maltose) per min. Solution viscosities were measured as previously described (Cote 1992), using a Brookfield LVTDV-1 digital viscometer. Methylation analysis was carried out using the method of Ciucanu and Kerek (1984) in which hydrolysis was accomplished using 2 M trifluoroacetic acid. The permethylated derivatives were analyzed by capillary GC-MS as the peracetylated aldononitrile (PAAN) derivatives (Seymour et al. 1975).

Results and discussion

Growth and dextranase production by *Penicillium* sp. strain NRRL 21969

Strains of *Penicillium* sp. that modify alternan produce no alternanase activity (Leathers et al. 2002a). However, we discovered that culture supernatants of *Penicillium* sp. strain NRRL 21969, germinated on alternan, do contain dextranase (Table 1). In fact, dextranase was produced more quickly and in higher yields on alternan than in cultures grown on dextran, despite the fact that growth on alternan was limited (Table 1). No dextranase activity was observed in cultures grown on glucose or incubated in the absence of a carbon source. This suggests that alternan or its partial hydrolysis products serve as non-metabolizable inducers for dextranase.

Modification of alternan by dextranase

To determine whether dextranase is directly related to alternan modification, alternan was treated with commercial dextranase from *Penicillium* sp. (Sigma) under conditions identical to those of the bioconversion process. Alternan modification is conveniently

Table 1 Growth and dextranase production by *Penicillium* sp. strain NRRL 21969

Carbon source	Dextranase (IU/ml)		Dry weight (mg/ml)
	(4 day culture)	(7 day culture)	(7 day culture)
None	<0.1	<0.1	<0.1
Glucose	<0.1	<0.1	8.8 ± 1.9
Dextran	<0.1	0.6 ± 0.1	3.5 ± 0.2
Alternan	0.7 ± 0.1	1.5 ± 0.2	1.5 ± 0.1

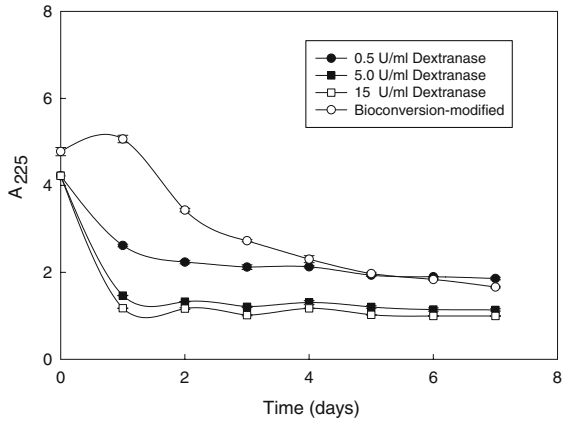

Fig. 1 Time course of alternan modification monitored by absorption at 225 nm. (•) 0.5 U/ml *Penicillium* sp. dextranase; (■) 5.0 U/ml *Penicillium* sp. dextranase; (□) 15 U/ml *Penicillium* sp. dextranase; (○) bioconversion modification

followed over time by monitoring the A_{225} of solutions (Leathers et al. 2002a, 2003). As shown in Fig. 1, dextranase added at 5 IU/ml or greater effected a rapid reduction in A_{225} to approximately 1. This is equivalent to the maximal amount of modification observed after 17 days in the bioconversion process (Leathers et al. 2002a).

The molecular weight distribution of native and modified alternan was analyzed by high pressure liquid size exclusion chromatography (HPLC SEC). As previously described, high molecular weight native alternan of approximately 10^6–10^7 is modified by bioconversion to a characteristic, lower molecular weight, heterodisperse form with peaks at approx $5–10 \times 10^5$ and $1–5 \times 10^4$ (Leathers et al. 2002a). In vitro treatment with dextranase results in a nearly identical product (Fig. 2). Dextranase-modified alternan also includes a minor low molecular weight peak eluting at about 20 min, subsequently shown to consist of free glucose and maltose or isomaltose. Presumably, these sugars are consumed in the bioconversion process in support of the limited spore germination. In addition, bioconversion-modified alternan was treated with dextranase, and showed no further modification (data not shown), indicating that modification is a type of limit digestion (i.e., all possible substrate sites are hydrolyzed).

Dextranase from *C. erraticum* was equivalent to that from *Penicillium* sp. for modification of alternan. Since this enzyme is considered generally regarded as

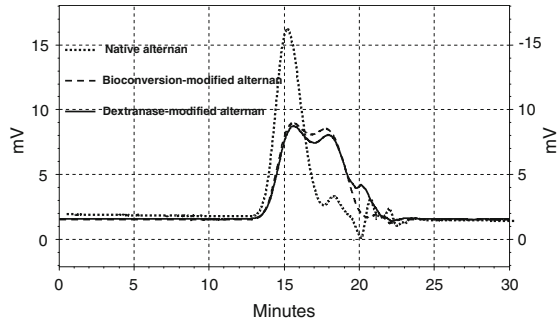

Fig. 2 Molecular weight distributions of native alternan, bioconversion-modified alternan, and dextranase-modified alternan, determined by HPLC size exclusion chromatography (HPSEC)

safe (GRAS), it may be useful in the modification of alternan for potential food uses.

Solution viscosity properties of modified alternan

Native alternan is highly soluble, although solutions of greater than 12–15% (w/v) are difficult to attain due to relatively high viscosity. Modified alternan can be dissolved in water to give solutions of at least 50% (w/v) (Leathers et al. 2003). The solution viscosity properties of bioconversion-modified alternan are far more similar to gum arabic than to native alternan (Leathers et al. 2002a). As shown in Fig. 3, dextranase-modified alternan is essentially identical to bioconversion-modified alternan.

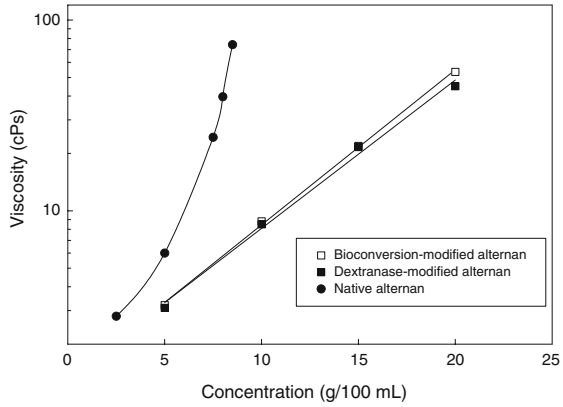

Fig. 3 Solution viscosities of solutions of native alternan, bioconversion-modified alternan and dextranase-modified alternan as a function of concentration. All solutions were tested at a shear rate of 39.6 s^{-1}

Table 2 Methylation analysis of alternan before and after treatment with endodextranase

Sample	2,3,4,6-tetra -*O*-Me	2,4,6-tri-*O*-Me	2,3,4-tri-*O*-Me	2,4-di-*O*-Me
Native (± SD)	7.7 ± 0.6	31.3 ± 0.5	49.3 ± 0.6	11.7 ± 1.3
Treated (± SD)	7.4 ± 1.6	35.5 ± 1.6	46.2 ± 0.9	10.8 ± 2.3
t-value	1.57	4.48	5.71	0.04
P	0.26	0.04	0.03	0.97

Mole percent of methylated products

$P < 0.05$ is significant

Methylation analysis of native and modified alternan

Alternan has long been considered to be resistant to dextranase because of its regular, alternating α-(1 → 6) and α-(1 → 3) linkage structure. However, it is possible that high molecular weight alternan includes limited regions with a variant structure that is susceptible to dextranase, perhaps short stretches of consecutive α-(1 → 6) linkages. Native and modified alternan were compared by methylation analysis for differences in structural details. As Table 2 shows, native alternan contained 31.3% linear 1,3-linkages and 49.3% linear 1,6-linkages, whereas modified alternan contained 35.5% linear 1,3-linkages and 46.2% linear 1,6-linkages. No significant difference in degree of branching was noted. This small but significant difference in linear linkages must occur in contiguous sequences that are hydrolyzed by endodextranase. Furthermore, the reduction in molecular weight and viscosity shows that these contiguous sequences must be distributed throughout the chains, rather than at the non-reducing ends.

Misaki et al. (1980) reported that alternan was hydrolyzed up to 7.3% by *Penicillium* endodextranase, but reported the products as glucose, isomaltose and other oligosaccharides. No changes in molecular weight or viscosity were noted. Their proposed structure for alternan placed the excess α-(1 → 6) linkages at branch points where they would presumably be resistant to endodextranase action. No linear sequences of α-(1 → 6)-linked regions were shown in their structure. Our results, however, indicate that at least some of the contiguous α-(1 → 6) linked sequences occur in regions that are susceptible to endodextranase hydrolysis, and that such hydrolysis changes the proportion of 1,3-linked and 1,6-linked linear linkages, but has no measurable effect on branching. Furthermore, the hydrolysis by endodextranase causes a significant change in the molecular weight distribution, sufficient to cause a noticeable loss of opalescence in aqueous solutions.

The action of endodextranase on alternan may be analogous to the limited susceptibility of pullulan to α-amylase. Pullulan is generally resistant to α-amylase because of its regular structure of α-(1 → 6) linked maltotriose subunits, as maltotriose is not a substrate for this enzyme. However, pullulan contains a minor percentage of maltotetraose subunits that are substrates for α-amylase (Catley 1970; Catley and Whelan 1971). The action of amylases reduces the molecular weight of pullulan in late cultures. In nature, such limited hydrolysis of extracellular polysaccharides may serve to reduce viscosity in response to environmental conditions, or perhaps to facilitate the detachment of microcolonies during the propagation of biofilms.

In summary, a novel modified alternan, previously produced by a bioconversion process, was produced in vitro using commercial dextranases, including a GRAS enzyme. Results suggest that alternan includes minor structural elements that vary from its primary linkage structure. These results yield new information on the structure of alternan and explain the basis of the bioconversion process. This provides a new, attractive method to produce modified alternan.

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